software, room temperature, ambient light conditions, and probe angle may influence the reflectance spectral values. Thus, all reflectance spectrophotometers should be independently validated in appropriate and known Fitzpatrick skin types under the environmental and investigator conditions of assessment to establish the mathematical-fitted equation between subjective and objective skin phototype. Given that the same light source was utilized throughout the studies, and reflected light spectra of commercial color and light/dark standards were validated daily, normalization of the reflectance spectral data with the dark current (0% transmission) and light source spectrum before each subject was not necessary for an accurate skin phototype assessment. Normalization methods may be useful, however, when comparison between instruments, light sources, investigators, or study locations is desired.

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Independent Evaluation of a Commercial Test for ''Autoimmune'' Urticaria in Normal and Chronic Urticaria Subjects

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TO THE EDITOR

Although the pathogenesis of the majority of chronic idiopathic urticaria (CIU) patients is unknown, approximately 40% of patients are proposed to have ''functional'' IgG autoantibodies against either the high-affinity IgE receptor (FcεRIα) or IgE as measured by the basophil histamine-releasing activity (HRA) assay (Ferrer and Kaplan, 2007). This assay involves incubating basophils from a healthy donor with a CIU patient's serum and measuring histamine release. A ''positive'' result is often judged in relation to basophil HRA levels obtained with serum from a non-CIU population. Some investigators have proposed that the presence of ''functional'' autoantibodies offers a pathogenic explanation for patients' symptoms or provides a rationale for treatment with immunomodulatory therapy such as cyclosporine (Sabroe and Greaves, 2006). However, the HRA assay, which is considered the ''gold standard'' (Ferrer and Kaplan, 2007) for ''functional'' autoantibodies, has limitations. Its performance depends on the unique characteristics of the healthy basophil donors (Eckman *et al.*, 2008). In addition, interlaboratory reproducibility of this test has not been possible to assess because of a lack of universally available standardized reagents.

An alternative line of investigation has shown that basophils from active CIU subjects manifest a suppressed IgE receptor-mediated histamine degranulation (Ferrer and Kaplan, 2007). We have stratified CIU subjects into responder (CIU R) and nonresponder (CIU NR) functional phenotypes based on the profile of *ex vivo* activation of their basophils by an optimal concentration (0.1 $\mu g\,ml^{-1}$) of polyclonal anti-IgE (Vonakis *et al.*, 2007). Subjects with $<10\%$ histamine release with anti-IgE stimulation were classified as CIU NR, whereas subjects with histamine release $\geqslant 10\%$ were classified as CIU R. This basophil classification remains consistent over the course of active disease (Eckman *et al.*, 2008). Interestingly, increased basophil IgE-receptor-mediated histamine release occurs in both groups as subjects enter disease remission (Eckman *et al.*, 2008). Using a previously identified immunoenzymometric assay (IEMA), we observed a similar prevalence and concentration of IEMA-detected autoantibodies in the CIU R, CIU NR, and non-CIU subject groups (Eckman *et al.*, 2008). We have previously reported that positive HRA presence occurs at a similar frequency in CIU R, CIU NR, and nonatopic, healthy (normal) subjects (Vonakis *et al.*, 2007). The positive HRA results we obtained in the non-CIU subjects have been challenged (Kaplan and Joseph, 2007).

The purpose of this study was to assess HRA activity in sera from CIU and non-CIU subjects by the CU Index test by IBT Laboratories (Lenexa, KS). Further, we examined the relationship of HRA activity to previously determined CIU basophil classification and the presence of IEMA-detected autoantibodies.

Following consent, whole-blood was collected for basophil and serology studies from subjects with a physician-determined diagnosis of CIU ($n=21$) or non-CIU controls ($n=22$; ages 18–65, 12 female, 10 male, 9 atopic, 13 nonatopic by history). None of the non-CIU subjects had any known autoimmune disease. Protocols were approved by the Johns Hopkins Institutional Review Board and the Western Institutional Review Board and were in adherence to the Declaration of Helsinki Principles (Eckman *et al.*, 2008). Basophil functional phenotyping studies were performed as described

Abbreviations: CIU NR, CIU non-responder; CIU R, CIU responder; CIU, Chronic idiopathic urticaria; HRA, Histamine release activity; IEMA, Immunoenzymetric assay

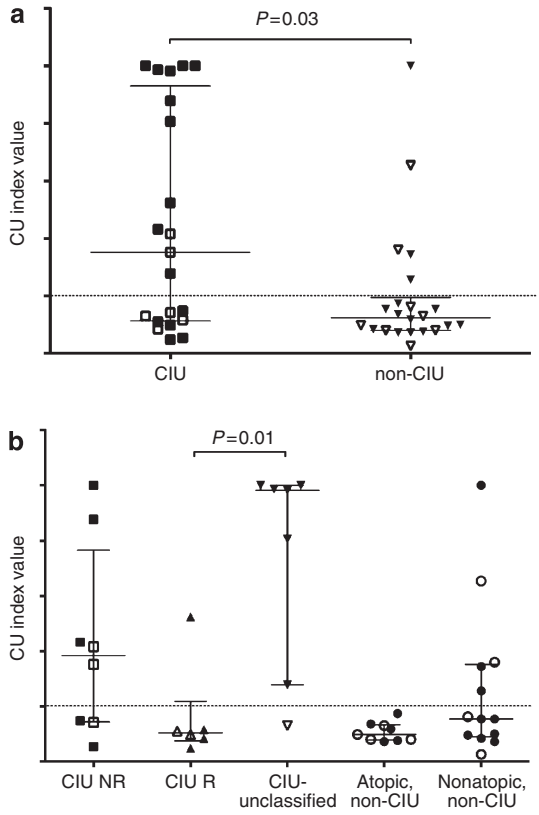

Figure 1. CU Index values in relation to subject grouping. (**a**) CU Index values for active CIU subjects and non-CIU subjects. (**b**) CU Index values are subdivided by CIU functional phenotype and disease state. Horizontal bars represent median values with 25–75% percentiles. Closed symbols are IEMA positive for IgG anti-FcεRIα and/or anti-IgE whereas open symbols are negative for both autoantibodies.

previously (Vonakis *et al.*, 2007). Subjects with a total blood leukocyte histamine content $<5\,ng\,ml^{-1}$ had extreme basopenia and were not able to be functionally classified (CIU unclassified). IgG anti-FcεRIα and IgG anti-IgE were measured by an immunoenzymometric assay as previously described (Eckman *et al.*, 2008). Coded sera were sent masked to IBT Laboratories for analysis in their CU Index test, which utilizes healthy donor basophils in an HRA assay format (Altrich *et al.*, 2008). According to IBT Laboratories, the CU Index cutoff is determined based on the percentage histamine release (of total histamine content) from donor basophils induced by CIU subject's serum divided by a donor-specific ''cut off'' percent histamine release, and then this ratio is multiplied by 10. This donor-specific ''cut off'' is determined by a statistical analysis of a dataset obtained from a panel of negative, non-CU sera. One basophil donor is used for all studies. A positive CU Index result was considered $\geqslant 10$ based on the HRA assay's reported reference range (0–50). Two-tailed Fisher's exact test was used for categorical analysis; whereas a two-tailed, Mann–Whitney *U*-test was used to compare the medians of different groups.

CU Index values were compared to subject classification (see Figure 1). Positive CU Index values were seen in both CIU and non-CIU subjects, and therefore, positive HRA presence is not exclusively observed in CIU subjects. The CU Index values were higher in the CIU group (median 17.6; $n=21$) as compared to the non-CIU group (median 6.2; $n=22$, $P=0.03$, Mann–Whitney test). Moreover, CIU subjects (57%) were significantly more likely to have a positive HRA test than non-CIU subjects (23%; $P=0.03$, Fisher's exact test). In CIU subjects, the presence of IEMA-detected IgG anti-FcεRIα and/or anti-IgE (see Figure 1a; closed symbols) was not associated with a positive CU Index ($P=0.33$; Fisher's exact test). Among CIU basophil phenotype subsets, CIU-unclassified subjects (median 17.6; $n=7$) had significantly higher CU Index values than the CIU R group (median 6.2; $n=6$; $P=0.01$, Mann–Whitney test). The CIU NR subjects' CU Index values were not significantly different from the levels in the other CIU groups. Between the non-CIU populations; nonatopic, non-CIU subjects ($n=13$) were more likely to have a positive test than atopic, non-CIU subjects ($n=9$; $P=0.05$, Fisher's exact test).

The significantly higher frequency and magnitude of CU Index values in the CIU group as compared to non-CIU group support the general hypothesis that serological factors in CIU subjects affect donor basophils differently than non-CIU subjects. However, these data raise questions about the clinical utility of HRA testing in the diagnosis and management of ''autoimmune'' CIU. The existence of strongly positive HRA levels in nonatopic, non-CIU patients is similar to what we observed in our previous studies (Vonakis *et al.*, 2007), and the reasons for this are not currently understood. In addition, there was no relationship between IEMA-detected autoantibodies and a positive CU Index. This lack of diagnostic specificity of the CU Index, and thus HRA, raises concerns about its general usefulness in providing clarity on the disease's pathogenesis to CIU patients. It may be argued that this lack of specificity may be due to the improper selection of the basophil donor for the HRA assay. It is not clear from the literature how many non-CIU subjects should be screened for low histamine release in choosing the correct basophil donor. However, we assumed that the basophil donor was optimized for this commercial assay. Because of the lack of consensus on the laboratory protocols for HRA, and thus the definition of ''chronic autoimmune urticaria'', it cannot be said definitively that the IBT assay used in this report makes the distinction between autoimmune and idiopathic urticaria. In addition, the IBT assay that did not establish a positive result was because of the IgG fraction of serum. The method for defining a positive value is different

from many other descriptions of the HRA assay. A lack of specificity also has been seen with the autologous serum skin test, which is another measure of ''functional'' autoantibodies in CIU. The autologous serum skin test is positive in up to 37% of non-CIU subjects (Guttman-Yassky *et al.*, 2007).

On a limited basis, HRA-positive CIU subjects have been reported to have more severe disease (Sabroe *et al.*, 2002) and to be more likely to have thyroid autoantibodies (Kikuchi *et al.*, 2003) than HRA-negative subjects. However, skin biopsies show similar pathology in HRA-positive versus HRA-negative subjects with CIU (Sabroe *et al.*, 1999; Ying *et al.*, 2002). More importantly, in making decisions about starting immunomodulatory medication such as cyclosporine in CIU patients, HRA measurement is not clearly needed, because the presence of ''functional'' autoantibodies is not correlated to clinical response (Morgan and Khan, 2008).

At the present time, available assays for autoimmunity are flawed and do not consistently assist clinicians in their understanding of CIU's pathogenesis. What is truly needed is a reproducible assay to advance the specific definition of autoimmune urticaria.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Polyclonality of Multiple Sporadic Basal Cell Carcinomas

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TO THE EDITOR

Basal cell carcinoma (BCC) is the most common cancer in humans. Very often patients are prone to develop multiple lesions at different body sites (Ramachandran *et al.*, 1999, 2001; and cited therein; Heitzer *et al.*, 2007). Recently, several studies have been performed to determine the clonal origin of BCC in patients with multiple lesions (Walsh *et al.*, 1998; Saldanha *et al.*, 2002; Shulman *et al.*, 2006). For instance, Shulman *et al.* (2006) performed loss of heterozygosity (LOH) analysis and X-chromosome inactivation studies to determine the clonal origin of multiple BCCs from patients exhibiting lesions at different anatomical locations. They reported that in all BCCs displaying LOH, the same allele was lost in multiple tumors from a given patient. Furthermore, an identical X-chromosome inactivation pattern was noted in certain tumors. Hence, they suggested that the majority of BCCs in an individual might originate from one single tumor cell clone, independent of the anatomical site and time of lesion occurrence. However, van Steensel and Frank (2006) questioned the theory of monoclonality of multiple BCCs. They stated that it is important to sequence the *PTCH* gene to prove monoclonality of multiple BCCs because *PTCH* mutations are a hallmark of BCCs. If identical *PTCH* mutations are present in distinct BCCs at different anatomical locations from individual patients with multiple lesions, it would indicate monoclonality. If not, then it would suggest that each tumor arises independently from a distinct initiated (progenitor) cell.